Forest Pest Management
Asheville Field Office

Report #87-1-20
August 1987

FINAL REPORT OF THE PILOT PROJECT TO ASSESS THE EFFECTIVENESS AND LONGEVITY OF GYPCHEK APPLIED TO LOW DENSITY GYPSY MOTH (*Lymantria dispar* L.) POPULATIONS IN VIRGINIA

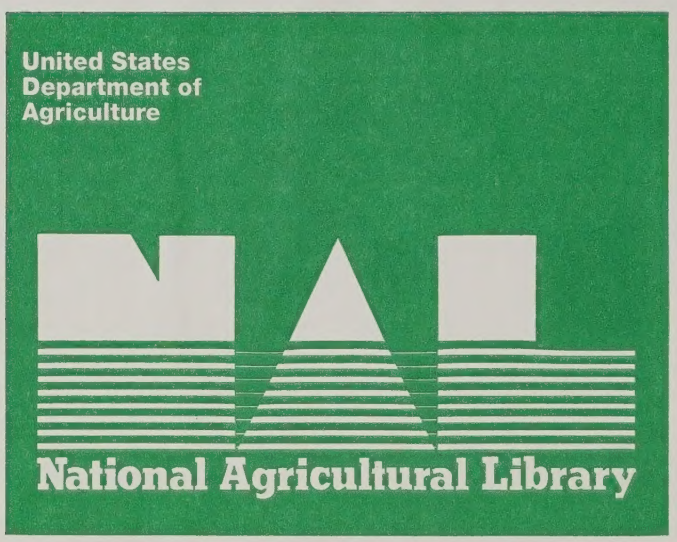
Prepared by: Cindy M. Huber
Entomologist

Caleb L. Morris
Retired Entomologist

John D. Podgwaite
Microbiologist

George W. Ryan
Statistician

Approved by: John H. Gherst
for: Harvey V. Toko
Staff Director
Forest Pest Management


United States
Department of
Agriculture
National Agricultural Library

FINAL REPORT OF THE PILOT PROJECT TO ASSESS THE EFFECTIVENESS AND LONGEVITY OF GYPCHEK APPLIED TO LOW DENSITY GYPSY MOTH (*Lymantria dispar* L.) POPULATIONS IN VIRGINIA

Cindy M. Huber[1], Caleb L. Morris[2], John D. Podgwaite[3], and George W. Ryan[4]

ABSTRACT

In 1985, GYPCHEK, the gypsy moth nucleopolyhedrosis virus product, was evaluated as a suppression tool against a low level gypsy moth population in Virginia. The objectives of the project were to evaluate the effectiveness of aerial application of GYPCHEK to low level populations of the gypsy moth and to determine whether the virus could be effective the year following application. Folic acid, an ultraviolet (UV) radiation protectant, was added to the spray mix to increase GYPCHEK's effectiveness. Results showed that GYPCHEK was effective the year of application, but this did not carry over into the following year. Since 1985, another UV protectant has been found that is proving to be quite effective and has replaced folic acid in the spray mix.

INTRODUCTION

Development of the gypsy moth nucleopolyhedrosis virus (NPV) product GYPCHEK, its advantages as a microbial pesticide, and the attendant problems associated with its use have been reviewed in some detail by Podgwaite (1984). It is clear that this product would be more efficacious if formulated in such a manner as to enhance its persistence on foliage. This is the general consensus of several investigators after extensive field testing of a variety of formulations and tank mixes over several years (Rollinson et al. 1965, Yendol and Hamlen 1973, Yendol et al. 1977, Wollam et al. 1978, Lewis et al. 1979, Lewis and Yendol 1981).

Further, GYPCHEK has only been evaluated as a direct suppression tool against moderate to dense gypsy moth populations. It would be desirable to use this product similarly in low to moderate populations with the added purpose of placing virus in generally infested areas prior to its natural occurrence and expression. This may lead to NPV epizootics occurring faster, temporally, than they normally would if these populations were left untreated.

---

[1]Entomologist, USDA Forest Service, FPM, Asheville, NC.

[2]Retired Entomologist, Virginia Department of Forestry, Charlottesville, VA.

[3]Microbiologist, USDA Forest Service, NEFES, Hamden, CT.

[4]Statistician, USDA Forest Service, FPM, Atlanta, GA.

Gypsy moth NPV is adversely affected by solar radiation. Several ultraviolet protectants, e.g., molasses and Shade, have been field tested with variable results. Several others have shown promise in the laboratory, but would not be cost effective in the field (Shapiro et al. 1983). Two products that have shown efficacy in the laboratory and that would be cost effective are the vitamins folic acid and riboflavin. Laboratory assays on both vitamins (tested as feed-grade products) have indicated high residual NPV activity (84-89%) after irradiation (Shapiro, unpublished data; Rollinson, unpublished data).

The purpose of this project was to test the effectiveness of a GYPCHEK - folic acid formulation in a low density gypsy moth population in Virginia. The project was coordinated with the gypsy moth IPM project in Maryland through Dr. Richard Reardon, USDA Forest Service, Morgantown, WV and Dr. John Podgwaite, USDA Forest Service, Hamden, CT.

OBJECTIVES

1. To evaluate the effectiveness of early (1st instar) aerial application of GYPCHEK to low level populations of the gypsy moth.

2. To determine whether the virus could be effective in low level gypsy moth populations for 2 years.

METHODS

Three 50-acre blocks of hardwood forest, located in northern Virginia (Figure 1), were treated with GYPCHEK on May 1 and again on May 8, 1985. A helicopter was used to aerially apply the virus.

Description of Formulation and Application Specifications

Formulation.--GYPCHEK, the laboratory-produced gypsy moth NPV, is a powder with 4% active ingredient (polyhedral inclusion bodies of gypsy moth nucleopolyhedrosis virus). It was registered by the EPA (Environmental Protection Agency) in 1978 and was given EPA Registration No. 27586-2. GYPCHEK is an extremely host specific virus which affects only the gypsy moth. It has been shown to have no adverse effects on humans, mammals, fish, birds, or nontarget insects. GYPCHEK also has no degrading effect on the environment in which it is applied.

The planned spray mix was GYPCHEK, 8L carrier (oil vehicle), 20% folic acid-feed grade (a UV screen), a sticker (B60A Rhoplex), and water (pH 7.0, approximately). The GYPCHEK, folic acid, and sticker were provided by the USDA Forest Service, Center for Biological Control of Northeastern Forest Insects and Diseases in Hamden, CT. The 8L carrier was obtained from Abbott Laboratories. The first application was made without the 8L carrier, because it did not contain an emulsifier and could not be used. A new batch of carrier was received in time to be used in the second application. The components of the spray mixes for two applications are given in Table 1.

Gypsy moth NPV is adversely affected by solar radiation. Several ultra-violet protectants, e.g., sunscreens and Shade, have been field tested with variable results. Several others have shown promise in the laboratory, but would not be cost effective in the field (Shapiro et al. 1983). Two products that have shown efficacy in the laboratory and that would be cost effective are the vitamins folic acid and riboflavin. Laboratory assays on both vitamins (tested as feed-grade products) have indicated high residual NPV activity (84-89%) after irradiation (Shapiro, unpublished data; Rollinson, unpublished data).

The purpose of this project was to test the effectiveness of a GYPCHEK - folic acid formulation in a low density gypsy moth population in Virginia. The project was coordinated with the gypsy moth IPM project in Maryland through Dr. Richard Reardon, USDA Forest Service, Morgantown, WV and Dr. John Podgwaite, USDA Forest Service, Hamden, CT.

OBJECTIVES

1. To evaluate the effectiveness of early (1st instar) aerial application of GYPCHEK to low level populations of the gypsy moth.

2. To determine whether the virus would be effective in low level gypsy moth populations for 2 years.

METHODS

Three 50-acre blocks of hardwood forest, located in northern Virginia (Figure 1), were treated with GYPCHEK on May 1 and again on May 8, 1985. A helicopter was used to aerially apply the virus.

<u>Description of Formulation and Application Specifications</u>

<u>Formulation</u>.--GYPCHEK, the laboratory-produced gypsy moth NPV, is a powder with 4% active ingredient (polyhedral inclusion bodies of gypsy moth nuclear-polyhedrosis virus). It was registered by the EPA (Environmental Protection Agency) in 1978 and was given EPA Registration No. 27586-2. GYPCHEK is an extremely host specific virus which affects only the gypsy moth. It has been shown to have no adverse effects on humans, mammals, fish, birds, or nontarget insects. GYPCHEK also has no degrading effect on the environment in which it is applied.

The planned spray mix was GYPCHEK, 8L carrier (oil vehicle), 40% folic acid-feed grade (a UV screen), a sticker (USDA Rhoplex), and water (pH 7.0, approximately). The GYPCHEK, folic acid, and sticker were provided by the USDA Forest Service, Center for Biological Control of Northeastern Forest Insects and Diseases in Hamden, CT. The 8L carrier was obtained from Abbott Laboratories. The first application was made without the 8L carrier, because it did not contain an emulsifier and could not be used. A new batch of carrier was received in time to be used in the second application. The components of the spray mixes for two applications are given in Table 1.

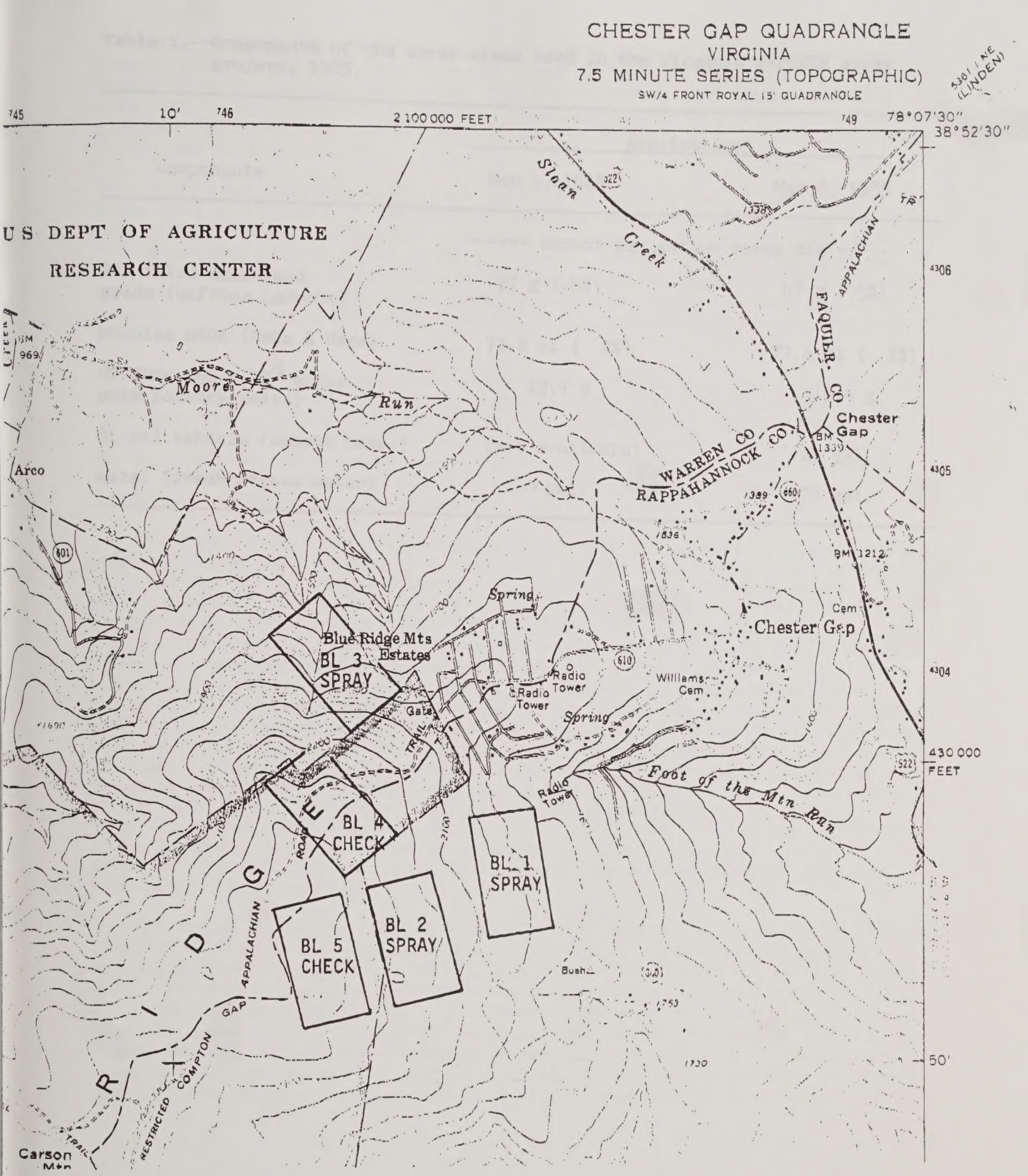


Figure 1.--Location of spray and check blocks for the 1985 GYPCHEK pilot project in Warren and Rappahannock Counties, Virginia.

Table 1.--Components of the spray mixes used in the Virginia GYPCHEK spray project, 1985.

Components	Application	
	May 1, 1985	May 8, 1985
	------ amount per gallon spray mix -----	
20% folic acid-feed grade (Hoffman LaRoche)	97 g (.5%)	97 g (.5%)
Rhoplex B60A (Rohm & Haas)	77.6 ml (2%)	77.6 ml (2%)
GYPCHEK (50×10^6 gypsy moth potency units)	19.4 g	19.4 g
8L oil vehicle (Abbott Labs.)	(not available)	.25 gal.
water (fresh stream water)	1.0 gal.	.75 gal.

Virus application.--GYPCHEK was applied at a dose of 50×10^6 gypsy moth potency units per acre. This dose was based on tests performed on gypsy moth larvae from the Virginia test area. Egg masses collected February 21, 1985 were sent to Dr. Podgwaite in Hamden, where the eggs were allowed to hatch and a sample of the larvae were bioassayed against GYPCHEK to determine field dose (Lewis and Rollinson 1978).

GYPCHEK was aerially applied at the rate of 1 gallon of finished spray per acre using a helicopter. The aircraft was equipped with flat fan nozzles of the appropriate size to produce a droplet size spectra of 100 to 150 microns, with a deposit pattern of 10 to 20 drops/cm^2. GYPCHEK was applied to three 50-acre forested blocks adjacent to the Shenandoah National Park in Warren and Rappahannock Counties, Virginia. Two check blocks, separated from the virus treated blocks by 1/8-1/4 mile, provided an "untreated" gypsy moth population for comparison.

Virus applications were planned for the evening between the hours of 6:30 and 8:00 p.m., weather permitting. Acceptable weather conditions for spraying were wind speed less than 6 mph and no rainfall predicted within the next 12 hours. A fire weather kit was used on the treatment site to measure wind speed, wind direction, temperature, and relative humidity. These data were taken and recorded prior to spraying and periodically during the spray operation. Table 2 summarizes weather conditions for both applications.

The first spray date was predicted based on a combination of first hatching date of larvae, leaf size, and availability of the Hamden crew. Larvae had been hatched for 8 days, and leaves were of ideal size (2" long) by May 1, 1985. Balloons were raised and cards were placed in the field for a 5:00 p.m. target spray time. Spraying began at 6:20 p.m. and finished at 8:15 p.m. Field crews finished picking up the spray cards and were out of the woods at 9:00 p.m. Weather conditions for the spray application were excellent. This was fortunate since the carrier, which was intended to reduce evaporation, could not be used as planned. The high humidity allowed us to omit the carrier and still have a good chance at adequate coverage. The aircraft spray nozzles stopped up immediately on the first spray run on block 3 due to a large amount of insect fragments from the virus preparation. This problem persisted in spite of continued filtering, until the last full load on block 1, which went on smoothly. The final spray run applied 15 gallons of spray on block 3, starting at the upper side and working down, until the tank was dry. Blocks 2 and 3 had very irregular spray coverage due to nozzle stoppage. Weather conditions during the first application were: temperature 81°, RH 52%, and wind speed less than 3 mph.

The second virus application on May 8, 1985 included a new batch of carrier supplied by Abbott and was characterized throughout with nozzle stoppage--apparently due to coagulation of the folic acid with the Abbott carrier. The pilot was able to apply only one-half load at a time. Spraying began at 5:45 p.m. and was completed at 8:15 p.m. Spray cards were retrieved by 8:30 p.m. Wind was 4 mph or less during the spray period; temperature was 74°F at start, 68°F at finish; and the relative humidity was 38%. Again excellent spray conditions, except for the 90% sunlight in the late afternoon. However, the addition of the carrier should have effectively reduced evaporation.

[illegible] GYPCHEK was applied at a [illegible] of [illegible] x 10[11] [illegible] acre [illegible] test area. [illegible] was cased at [illegible] from the Virginia test area. Egg masses [illegible] January 21, 1985 [illegible] to Dr. Podgwaite in Hamden, where [illegible] allowed to hatch and [illegible] of the larvae were bioassayed against [illegible] [illegible] and Rollinson 1976).

[illegible] was aerially applied at the rate of 1 gallon of [illegible] using a helicopter. The aircraft was equipped with [illegible] appropriate size to produce a droplet size spectra of [illegible] a deposit pattern of [illegible] to 20 drops/cm². GYPCHEK was applied to [illegible] treated blocks adjacent to the Shenandoah [illegible] Counties, Virginia. Two check blocks [illegible] blocks by [illegible] size, provided an "untreated" [illegible] population.

[illegible] applications were planned for the morning between the hours [illegible] p.m., weather permitting. Acceptable weather conditions for spraying [illegible] less than 6 mph [illegible] within the [illegible] A five weather kit was used to [illegible] wind direction, temperature, and relative humidity [illegible] and recorded prior to spraying and periodically [illegible] cation. Table 2 summarizes weather conditions for both applications.

First spray date was predicated on a combination of [illegible] larvae, leaf size, and availability of the flight crew. [illegible] for 8 days, and leaves were of ideal size [illegible] Balloons were released and cards were placed in the field [illegible] spray time. Spraying began at [illegible] p.m. and finished at [illegible] crew finished picking up the spray cards and [illegible] p.m. Weather conditions [illegible] spray [illegible] fortunate since the carrier, which was intended to reduce evaporation, [illegible] be used as planned. The high humidity [illegible] have a good chance at adequate coverage. [illegible] spray nozzle [illegible] immediately on the first spray run on block [illegible] large amount [illegible] fragments from the [illegible] This problem persisted in [illegible] until the last [illegible] The final [illegible] gallons of [illegible] at the upper end and working down [illegible] very irregular spray [illegible] during the first application [illegible] temperature [illegible] less than 3 mph.

The second application [illegible] May 8, 1985 [illegible] new batch of [illegible] by Abbott and [illegible] due to [illegible] the [illegible] was able to apply only one-half load [illegible] Spraying [illegible] completed at [illegible] p.m. [illegible] mph or less during the spray [illegible] temperature [illegible] 68° [illegible] and [illegible]

[illegible] reduced evaporation.

Table 2.--Weather information for the two GYPCHEK sprays in Virginia, 1985.

Time PM	Block #	Temp °F	Wind Speed mph	RH %	Notes
First Application - May 1, 1985					
5:55	3	74	<2	36	Began spraying load 1.
6:00					Stopped spraying due to clogged nozzles.
6:35	3	73	<2	39	Started spraying again.
6:44					Finished spraying/filled tank.
6:50	2	70	2-2.5	48	Began spraying load 2.
7:15					Finished spraying/filled tank.
7:35	1	68	0-3.5	54	Began spraying load 3.
7:55					Finished spraying/filled tank.
8:03	3	68	2.5-4	54	Sprayed 15 gallons on the lower half of block 3, because the pilot felt he missed it on the first round.
8:10					Finished spraying.

General weather conditions:

It was overcast and a front was moving in.
It rained the following day, May 2.

Table 2.--con't

Second Application - May 8, 1985

5:30	3	68	<2	34	Began spraying.
5:45					Stopped spraying due to clogged nozzles.
6:15					Resumed spraying, but had to make two trips (2 loads) to spray each block.
6:45	2	67	<2	41	Began spraying.
7:00					Stopped to get second load.
7:10					Resumed spraying.
7:20					Finished spraying.

Block 1 was sprayed between 7:30 and 8:00, but no weather information was collected, because the weather person was collecting spray cards.

General weather conditions:

It was mainly clear, with a few clouds and quite calm. No rain.

Spray deposit assessment.--Spray distribution within the treatment areas was determined using Kromkote spray deposit cards within the spray blocks. Two cards were placed in open areas on the ground in each of the four corners of the spray blocks. Cards were also put out in the check blocks to monitor drift of the spray.

The spray cards were analyzed for presence or absence of spray deposit and droplet size. The results are shown in Table 3. The droplet spectra of the second spray was characterized using the D-max method to determine the volume median diameter (VMD). The VMD is the droplet diameter which divides the spray volume into two equal parts--50% of the spray volume is in droplet sizes below the VMD and 50% is above the VMD. A spread factor of 2 was used in this determination (obtained from Harry Hubbard, NEFES, Hamden, CT). The VMD could not be determined for the first spray, because there is no known spread factor for the formulation used, where the 8L carrier was absent.

Evaluation of Treatment Effectiveness

Virus incidence and population level data were used to determine the effectiveness of the spray project. These data were collected from 20 1/40-acre sample plots within each treatment block. Figure 2 shows the arrangement of sample plots within the blocks.

Virus incidence.--Pre-spray virus level (% infection) of the gypsy moth population in the study area was determined by Dr. John Podgwaite from larvae reared from egg masses collected in February 1985.

Changes in larval virus level following treatment were determined by analyzing larvae collected from the sample plots. All preferred host trees in the sample plots were burlap banded early in May 1985. When 50% of the larvae were in the 3rd instar (week of May 20), weekly larval collections were initiated. Each week, subsequent instars were collected from under the burlap bands and sent to Dr. Michael Ma, University of Maryland, for analysis using an enzyme linked immunosorbent assay (ELISA). The ELISA analysis was done only on larvae collected in 1985.

Population changes.--Gypsy moth population changes in the treated and check blocks were compared to evaluate the effectiveness of the GYPCHEK treatment. The populations were monitored using pre- and post-spray egg mass counts and weekly larval counts. All counts were taken from the 20 1/40-acre plots in each block.

In early April 1985, pre-spray egg mass density was determined by counting all egg masses present on trees, rocks, and branches on the ground within each 1/40-acre plot. The same procedure was repeated in the winter of 1985 and again in 1986.

Weekly larval counts (indicating number dead and alive) under all burlap bands were taken for 8 weeks in both 1985 and 1986.

Statistical analyses.--Statistical procedures (primarily analysis of variance [ANOVA]) were used to analyze egg mass data, larval counts, and percent and amount of virus as follows:

Table 3.--Spray deposit information for the GYPCHEK project in Virginia, 1985.

Block #	Plot Cluster	Presence or Absence of Spray Deposit				VMD[a]
		May 1, 1985		May 8, 1985		
		Card 1	Card 2	Card 1	Card 2	May 8, 1985
1	1-4	+[b]	+	-[c]	+	91
	5-8	+	+	+	+	114
	13-16	-	-	-	-	d
	17-20	-	-	-	-	d
2	1-4	+	+	+	+	114
	5-8	-	-	+	+	160
	13-16	+	+	+	-	114
	17-20	+	+	-	-	d
3	1-4	+	+	+	+	148
	5-8	+	+	+	+	57
	9-12	-	-	+	-	114
	13-16	+	+	+	+	125
4	1-4	-	-	-	-	
	17-20	+	+	-	-	
5	5-8	-	-	-	-	
	17-20	-	-	-	-	

[a]Average for the two cards.

[b] + indicates there were spray droplets on the card.

[c] - indicates there were no spray droplets on the card.

[d]No spray droplet, therefore VMD could not be calculated.

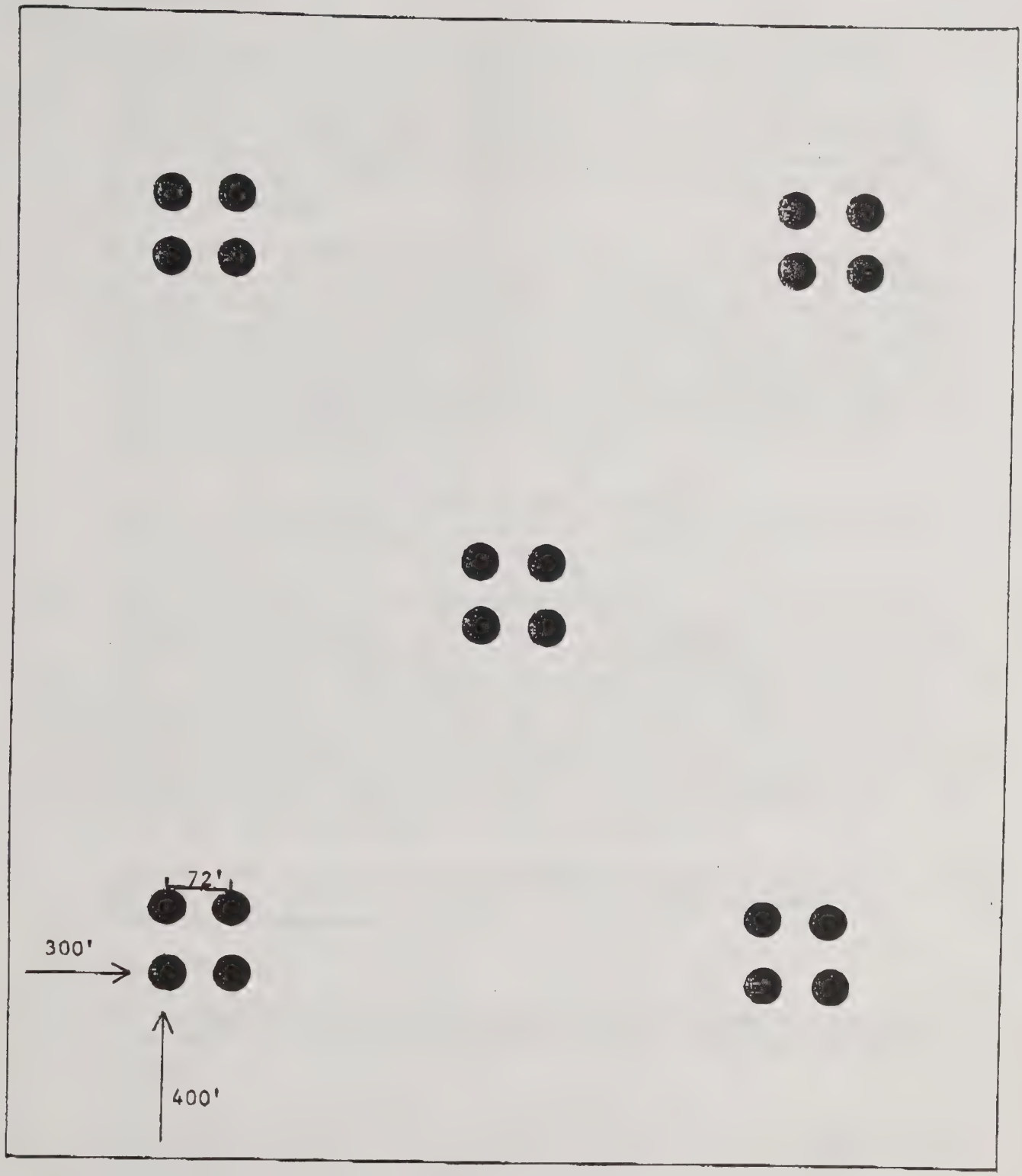


Figure 2.--Arrangement of the 20 1/40-acre sample plots within the 50-acre block.

1. Egg Mass Data

The analysis of this data determines how effective the GYPCHEK treatment was in the gypsy moth population. To determine the treatment's effectiveness, the analysis must concentrate on the magnitude of change, pre- to post-treatment, in egg mass numbers between spray and check blocks. Gerry Walton, USDA Forest Service, Hamden, CT, recommended some form of ratio estimator be used to analyze this data.

On account of differing population levels between the check and GYPCHEK spray plots, the data must be "adjusted" prior to analysis. When the data was adjusted, the adjustment was made only in the GYPCHEK spray blocks, because the population levels in these blocks are less than the levels in the check plots. In each GYPCHEK spray block, the pre-treatment reading in each plot is multiplied by the ratio of the average pre-treatment check plot reading to the average pre-treatment GYPCHEK plot reading to get the adjusted GYPCHEK pre-treatment plot reading.

The adjustment for the post-treatment reading for each GYPCHEK plot is done by multiplication by the ratio of the adjusted to unadjusted pre-treatment readings for that plot.

The whole purpose of the adjustment plan then in this manner is to handle the difference in pre-treatment population levels between GYPCHEK spray and check plots. As noted previously, ratio estimators were used to analyze the data. By using such estimators, similar adjustments can be effected by means of the estimates themselves. However, because the population levels in some blocks were so low, the technique described to adjust the data was used to avoid the potential problems of unacceptably high variation incurred through the division by small numbers, which is precisely the type of mathematical operation taking place with ratio estimators.

Once the data were adjusted, the dependent variable used in the ANOVA was the ratio (post-treatment reading minus pre-treatment reading)/pre-treatment reading.

2. Larval Counts

The analysis of larval counts was intended to answer the question "Were there fewer larvae in GYPCHEK sprayed blocks than in check blocks?"

Since the total larval count is proportional to the total number of egg masses, the data in each block (GYPCHEK as well as check) may be divided by the number of egg masses (pre-treatment) to adjust for differences in population levels.

The dependent variable used in the ANOVA was the square root of each observation adjusted as noted above; i.e., the square root transformation, a common variance stabilizing technique, was used. Also analyzed were the logarithm of each observation and the untransformed observation. The almost totally uniform results from the ANOVA's within each run for each of these variables indicates the

validity of the statistical procedure. That is, the data apparently adheres to the assumptions of analysis of variance as shown by the consistency of the results.

3. Amount and Percent of Virus

The ELISA analysis was done on the 1985 larval counts to determine if there are differences in percent infectivity and amount of virus between the GYPCHEK treated and the check blocks.

Since the variables are measurements (readings) and percentages, respectively, no adjustments were needed in the data. A logarithmic transformation was made on each reading (on account of the highly "skewed" distribution of the virus readings) and used as the dependent variable in the ANOVA. As in the larval count analysis, the actual variable itself and the square root transformation were also analyzed. For the percent infectivity data, the familiar and frequently used inverse sine square root function was taken as dependent variable.

RESULTS

Comparison of pre- (1984) and post-treatment (1985) egg mass densities shows an increase in egg masses in both the GYPCHEK treated and check blocks (Table 4). When the post-treatment figures are adjusted to equalize the differences in population levels that existed prior to treatment, there remains a statistically significant difference at the 1% level between the GYPCHEK and check blocks: $F = 8.35$, $p < .01$ when analyzed by block; $F = 17.26$, $p < .01$ when analyzed by treatment ($p < .01$ indicates statistical significance at .01 level of significance). The check blocks had significantly more egg masses/acre than the GYPCHEK treated blocks. It appears that GYPCHEK helped keep the gypsy moth population at a low level the year of treatment. A Duncan's multiple range test on the five 1985 block means shows one of the check blocks (5) to be significantly greater than the GYPCHEK block means, although the other check block (4) is not.

The 1986 egg mass data shows a dramatic increase in egg mass density in both the treated and check blocks (Table 4). Also, the population growth in the spray blocks (1, 2, and 3) exceeded that in check block 4. Apparently, GYPCHEK is no longer effective after 2 years. The F value of 2.67 for treatment in the ANOVA is not statistically significant at the .05 level. When analyzed by block, the F of 4.59 is significant at the .01 level, but this is due again (see results of Duncan's test in Table 4) to the large reading in block 5, a check block.

The larval count data from 1985 and 1986 substantiate these results (Table 5). In both years, there were significantly more larvae per 1/40-acre plot in the check blocks than in the GYPCHEK treated blocks. The difference is greater and more meaningful biologically in 1985. The 1986 figures, although statistically different, are essentially the same biologically.

No larval mortality was observed directly (dead larvae under burlap bands) in 1985. In 1986, dead larvae were found. There is a statistically significant difference between larval mortality in the GYPCHEK treated blocks (2%) and the check blocks (5%), but biologically there is no difference between the two (Figure 3).

Table 4.--Comparison of pre- and post-treatment egg mass densities by block and treatment.

Block	# Egg Masses/Acre (Pre-treatment-1984) (Mean ± SE)	# Egg Masses/Acre (Post-treatment-1985) (Mean ± SE)	# Egg Masses/Acre (Post-treatment-1986) (Mean ± SE)
1	60 ± 33.53	346 ± 112.06 A[1]	8,280 ± 1,620.4 A
2	48 ± 15.24	198 ± 67.53 A	12,096 ± 2,092.0 A
3	53 ± 14.67	294 ± 66.06 A	13,312 ± 1,447.6 A
4	178 ± 47.01	1,312 ± 227.81 A	12,220 ± 968.8 A
5	96 ± 14.62	2,932 ± 315.94 B	31,194 ± 3,040.4 B
Treatment			
GYPCHEK	53.67 ± 13.0	279.32 ± 48.68 A	11,228.0 ± 1,026.8 A
Check	137.00 ± 25.17	2,122.00 ± 231.91 B	21,708.0 ± 2,188.4 A

[1]Results from Duncan's multiple range test. Different letters in the column indicate significant differences between treatment at the 1% level. The data were adjusted for analysis to equalize differences in population levels prior to treatment.

Table 5.--Comparison of larval populations over the feeding period in GYPCHEK treated and check areas, 1985 and 1986.

Treatment	# Larvae per 1/40-acre Plot 1985 (Mean ± SE)	# Larvae per 1/40-acre Plot 1986 (Mean ± SE)
GYPCHEK	7 ± .5 A[1]	36 ± 1.7 A
Check	42 ± 2.4 B	38 ± 3.4 B

[1]Different letters in the column indicate significant differences between treatments at the 1% level (for 1985, $F = 15.94$, $p < .01$; for 1986, $F = 47.61$, $p < .01$). A square root transformation was used on the data prior to analysis.

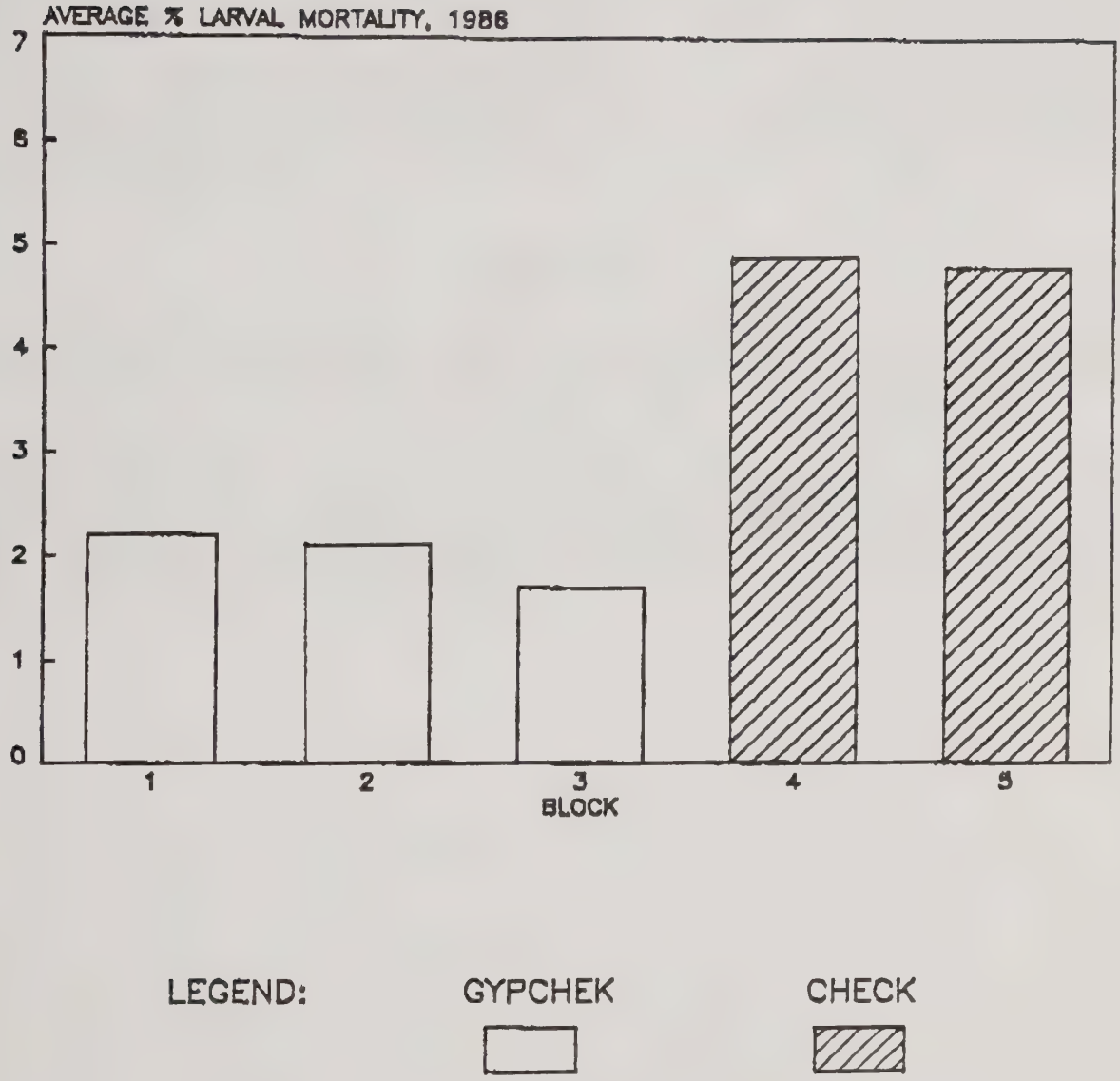
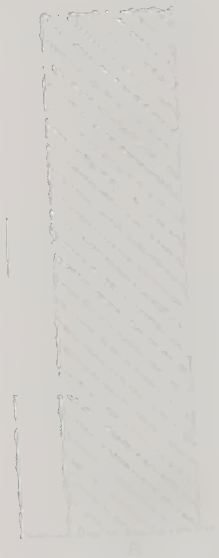


Figure 3.--Gypsy moth larval mortality in 1986, one year after GYPCHEK treatment, in Virginia.



In 1985, no observable defoliation occurred, but in 1986, there was obvious defoliation from gypsy moth. There appears to be a significant difference between defoliation in the GYPCHEK treated and check blocks (Figure 4A); but when percent defoliation is adjusted to account for the original differences in gypsy moth populations, there is no significant difference between the two treatments (Figure 4B).

The ELISA technique was used to evaluate the presence of NPV in the larvae in 1985. Analysis of these results showed no significant differences in percent of larvae with associated virus between the GYPCHEK treated and check blocks (Table 6). Likewise, there was no difference in the amount of NPV per larvae between GYPCHEK treated and check blocks. Because of problems associated with the ELISA technique and the fact that no difference in virus incidence or amount were seen in 1985, it was decided that the ELISA technique would not be used in 1986.

CONCLUSIONS

The results of this project indicate that GYPCHEK (with folic acid) applied to low level gypsy moth populations can maintain populations at low levels the year of treatment, but the effect does not extend into the second year.

Since 1985, when this project was initiated, an even more effective UV screen has been found. Orzan, a by-product of the tree pulping process, was tested in 1986 in Maryland and was found to be superior to folic acid as a sun screen. Development of the GYPCHEK/Orzan combination holds more promise for GYPCHEK's usefulness, especially against low level populations of the gypsy moth.

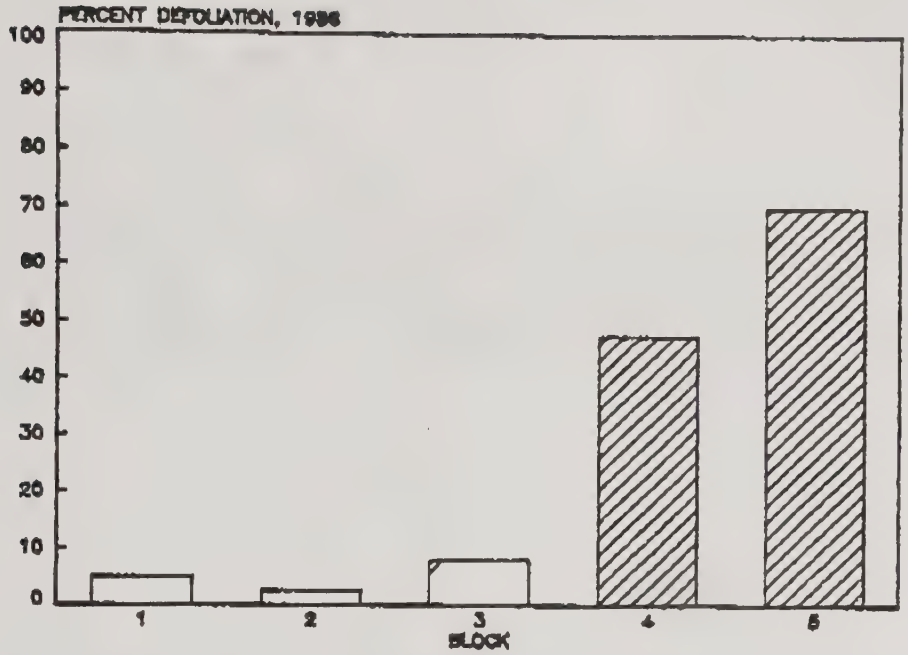


Figure 4A.--Defoliation by gypsy moth in 1986, one year after GYPCHEK treatment.

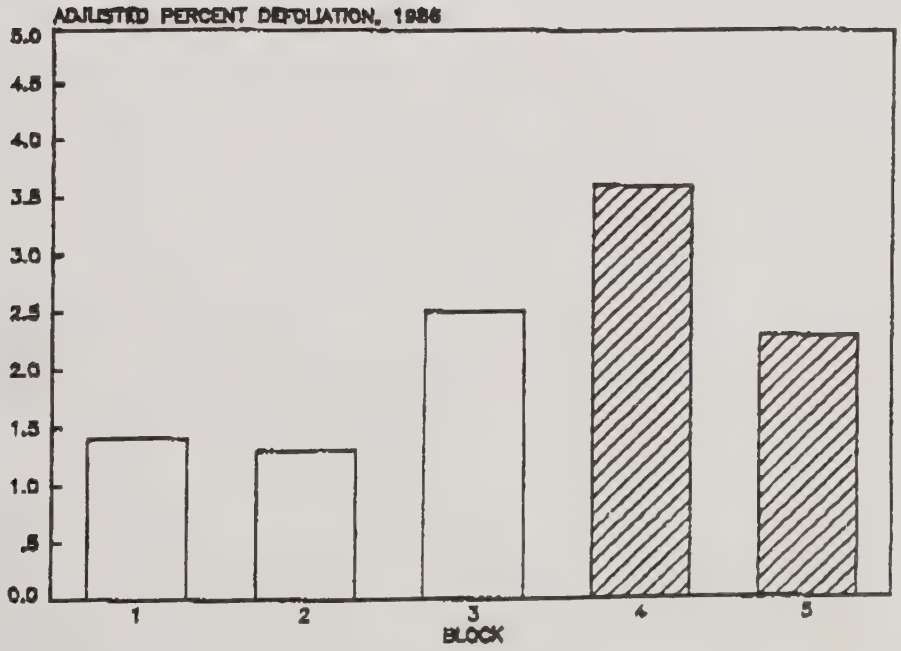


Figure 4B.--Defoliation by gypsy moth in 1986 after being adjusted to reflect the differences in population levels prior to GYPCHEK treatment in 1985.

Table 6.--Comparison of percent virus contaminated larvae in 1985 and the amount of virus associated with the infected larvae.

Treatment	Percent Larvae with Virus (Mean ± SE)	Mean NPV (g)/Infected Larvae ± SE
GYPCHEK	34 ± .04 A[1]	364.01 ± 51.94 A
Check	36 ± .05 A	322.60 ± 97.98 A

[1]Same letter in column indicates no significant differences between treatments. The respective F values for each of these differences is .08 (.7890) and 4.18 (.0868). The significance probabilities (the quantities in parentheses) being larger than .05 indicate that the observed differences are not statistically significant.

LITERATURE CITED

Lewis, F.B.; W.D. Rollinson. Effect of storage on the virulence of gypsy moth nucleopolyhedrosis inclusion bodies. J. Econ. Entomol. 71:712-722: 1978.

Lewis, F.B.; Reardon, R.C.; Munson, A.S.; Hubbard, H.B., Jr.; Schneeberger, N.F.; White, W.B. Observations on the use of GYPCHEK. Res. Pap. NE-447. Broomall, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station. 1979. 8 pp.

Lewis, F.B.; Yendol, W.G. Gypsy moth nucleopolyhedrosis virus: Efficacy. In: The Gypsy Moth: Research toward integrated pest management (C.C. Doane and M.L. McManus, eds.). pp. 503-512. Tech. Bull. 1584. Washington, DC: U.S. Department of Agriculture, Forest Service, Science and Education Agency. 1981.

Podgwaite, J.D. GYPCHEK: Past and future strategies for use. In: Proc. Symp. Microbial Control of Spruce Budworms and Gypsy Moths. pp. 91-93. Gen. Tech. Rpt. NE-100. Broomall, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station. 1984. 75 pp.

Rollinson, W.D.; Lewis, F.B.; Waters, W.E. The successful use of a nuclear polyhedrosis virus against the gypsy moth. J. Invertebr. Pathol. 7:515-517. 1965.

Shapiro, M.; Agin, P.P.; Bell, R.A. Ultraviolet protectants of the gypsy moth (Lepidoptera: Lymantriidae) nucleopolyhedrosis virus. Environ. Entol. 12:982-985. 1983.

Wollam, J.D.; Yendol, W.G.; Lewis, F.B. Evaluations of aerially-applied nuclear polyhedrosis virus for suppression of the gypsy moth, Lymantria dispar L. Res. Pap. NE-396. Broomall, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station. 1978. 8 pp.

Yendol, W.G.; Hamlen, R.A. Ecology of entomogenous viruses and fungi. Ann. N.Y. Acad. Sci. 217:18-30. 1973.

Yendol, W.G.; Hedlund, R.C.; Lewis, F.B. Field investigations of a baculovirus of the gypsy moth, Lymantria dispar L. J. Econ. Entomol. 70:598-602. 1977.